

Altered distribution of platelet-activating factor-acetylhydrolase activity between LDL and HDL as a function of the severity of hypercholesterolemia

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Abstract Platelet-activating factor-acetylhydrolase (PAF-AH) is a lipoprotein-associated phospholipase A₂ capable of hydrolyzing platelet-activating factor (PAF) and oxidatively modified phospholipids. We studied the plasma- and lipoprotein-associated PAF-AH activity in patients with primary hypercholesterolemia. Thirty-eight unrelated patients with heterozygous familial hypercholesterolemia (HeteroFH), five patients with homozygous FH (HomoFH), and 33 patients with primary non-FH hypercholesterolemia (NonFH) participated in the study. In all patient groups the plasma PAF-AH activity was significantly elevated compared with 33 normolipidemic controls, the HomoFH having the highest and the NonFH patients showing the lowest enzyme activity. Gradient ultracentrifugation studies showed that this increase is not only due to the elevation in the plasma LDL but also to the increase in the PAF-AH activity associated with each LDL subfraction, being more profound in the small-dense LDL₅. Unlike LDL, no difference in the HDL-associated PAF-AH activity was observed among all groups. Consequently, an altered distribution of enzyme activity among apolipoprotein B (apoB)- and apolipoprotein A-I (apoA-I)-containing lipoproteins is observed in hypercholesterolemic patients, resulting in a significant decrease in the ratio of the HDL-associated PAF-AH to the total plasma enzyme activity compared with controls. This reduction is proportional to the increase of the plasma LDL-cholesterol (LDL-C) levels and consequently to the severity of the hypercholesterolemia. Thus, the ratio of HDL-associated PAF-AH-total plasma enzyme activity may be useful as a potential marker of atherogenicity in subjects with primary hypercholesterolemia.—Tsimihodimos, V., S.-A. P. Karabina, A. P. Tambaki, E. Bairaktari, G. Miltiadous, J. A. Goudevenos, M. A. Cariolou, M. J. Chapman, A. D. Tselepis, and M. Elisaf. Altered distribution of platelet-activating factor-acetylhydrolase activity between LDL and HDL as a function of the severity of hypercholesterolemia. *J. Lipid Res.* 2002. 43: 256–263.

Supplementary key words familial hypercholesterolemia • low density lipoprotein • lipoprotein subspecies • high density lipoprotein • PAF-acetylhydrolase • polygenic hypercholesterolemia

Platelet-activating factor (PAF) is a potent lipid mediator involved in inflammatory diseases (1) that may equally be implicated in atherogenesis (2). In plasma, PAF is rapidly hydrolyzed and converted to lyso-PAF by PAF-acetylhydrolase (PAF-AH) (EC 3.1.1.47), a Ca²⁺-independent phospholipase A₂ (3). PAF-AH has a marked preference for phospholipids with short chain moieties at the *sn*-2 position and, with the exception of PAF, it can equally hydrolyze oxidized phospholipids containing an *sn*-2 polyunsaturated fatty acyl residue (4). Such phospholipids are formed during oxidative modification of LDL and potentially contribute to the biological activities of oxidized LDL (5, 6). PAF-AH could therefore act as a potent anti-inflammatory and anti-atherogenic enzyme. However, during the hydrolysis of oxidized phospholipids, PAF-AH liberates bioactive oxidized FFAs (7) and generates lysophosphatidylcholine, both of which are implicated in the biological actions of oxidized LDL (8). Thus, PAF-AH could equally play a pro-inflammatory and pro-atherogenic role. Consequently, the role of PAF-AH in inflammation and in atherogenesis is currently the subject of controversy.

PAF-AH in plasma circulates in active form complexed to lipoproteins, particularly with LDL and HDL (9). Thus, PAF-AH is also denoted as lipoprotein-associated phospholipase A₂ (8). In normolipidemic plasma (10) as well as in plasma from patients with heterozygous familial hypercholesterolemia (HeteroFH) (11), the distribution of

Abbreviations: BCA, bicinchoninic acid; HeteroFH, heterozygous familial hypercholesterolemia; HomoFH, homozygous familial hypercholesterolemia; LSD, least significant difference; NonFH, polygenic hypercholesterolemia; PAF, platelet-activating factor; PAF-AH, PAF-acetylhydrolase.

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PAF-AH among LDL particle subspecies is heterogeneous, and the majority of enzyme activity is associated with small-dense LDL particles. The relationship of plasma PAF-AH activity to circulating levels of LDL in familial hypercholesterolemia is a subject of debate. We previously reported that the LDL subfractions of heteroFH patients carry significantly higher PAF-AH activity as compared with normolipidemic subjects, thereby resulting in a higher plasma enzyme activity (11). In contrast, other investigators showed that in homozygous FH (HomoFH) LDL-associated PAF-AH activity is about 3-fold lower than in normolipidemic control subjects, whereas the enzyme activity in plasma is in the normal range (12). The factors responsible for the differences in the LDL-associated and in plasma PAF-AH activity among HeteroFH and HomoFH patients remain indeterminate (13, 14). Furthermore, the relative distribution of the enzyme between atherogenic LDL and anti-atherogenic HDL may be of special relevance to the cardiovascular risk associated with this phospholipase.

We undertook the present study to compare, under the same experimental conditions, *i*) the plasma- and lipoprotein-associated PAF-AH activity in HomoFH patients with that in a HeteroFH population as well as in patients presenting with primary non-FH hypercholesterolaemia (NonFH), and *ii*) the relative distribution of PAF-AH associated with atherogenic LDL versus anti-atherogenic HDL particles as a function of the severity of hypercholesterolemia.

MATERIALS AND METHODS

Study population

Five unrelated HomoFH patients, 38 unrelated HeteroFH patients, and 33 NonFH patients attending the Lipid Clinic at the University Hospital of Ioannina participated in the study. The diagnosis of familial hypercholesterolemia was made according to recently proposed criteria (15) and was confirmed by appropriate genetic analysis. Patients with NonFH were selected from those followed up in our Lipid Clinic. These patients did not exhibit tendon xanthomata or a strong family history of dyslipidemia. No tendon xanthomata were observed in their first degree relatives either. Furthermore, appropriate genetic analysis, which included sequencing of all exons of the LDL receptor, excluded the existence of familial hypercholesterolemia in this patient group. No patient had any evidence of cardiovascular disease by history, clinical examination, or electrocardiogram. Thirty-three healthy volunteers with no history of hypercholesterolemia or coronary artery disease were used as controls. None of the participants were taking lipid-lowering drugs or any other medication known to affect lipid metabolism, including hormonal therapy, during the last 12 weeks. Individuals with hypertension (blood pressure >140/90 mmHg on repeated measurements), diabetes mellitus (fasting blood glucose >126 mg/dl), obesity (body mass index >30 kg/m²) or thyroid, hepatic, or renal disease as well as subjects known to ingest more than two alcoholic drinks daily or taking vitamin supplements were excluded from the study. All patients were advised on a standard low-cholesterol (<300 mg per day), low fat (less than 30% of total calories) diet and were asked to adhere to this diet for at least 6 weeks before sample collection. Blood samples were obtained after a 14 h overnight fast. All study participants gave informed consent for the investigation, which was approved by the Ethical Committee of the University Hospital of Ioannina.

DNA analysis

Whole blood was collected from all the subjects investigated and DNA was extracted from white blood cells. PCR was carried out using 50 ng DNA isolated from each individual. DNA analysis for the LDL receptor gene was performed as described previously (16).

Preparation of lipoprotein subfractions

Lipoproteins were fractionated by isopycnic density gradient ultracentrifugation using a Beckman SW 41 Ti rotor at 40,000 rpm for 44 h in a Beckman L7-65 ultracentrifuge at 15°C, as described by Chapman et al. (17). After ultracentrifugation, 24 fractions, 0.4 ml each, were collected and analyzed for their protein content. Subsequently, equal volumes of certain gradient fractions were pooled to constitute the lipoprotein subfractions, as follows: fractions 1 and 2 (VLDL+IDL; $d < 1.019$ g/ml); 3 and 4 (LDL-1; $d = 1.019$ – 1.023 g/ml); 5 and 6 (LDL-2; $d = 1.023$ – 1.029 g/ml); 7 and 8 (LDL-3; $d = 1.029$ – 1.039 g/ml); 9 and 10 (LDL-4; $d = 1.039$ – 1.050 g/ml); 11 and 12 (LDL-5; $d = 1.050$ – 1.063 g/ml); 13 to 16 (HDL-2; $d = 1.063$ – 1.100 g/ml); 17 to 22 (HDL-3; $d = 1.100$ – 1.167 g/ml); 23 and 24 (VHDL; $d = 1.167$ – 1.190 g/ml). All subfractions were extensively dialyzed, in a 10 mM PBS buffer containing 2 mM EDTA, pH 7.4 at 4°C, filter-sterilized, and maintained at 4°C under nitrogen until analysis. Under these storage conditions, no oxidation has been reported to occur (18).

PAF-AH assay

PAF-AH activity was measured by the trichloroacetic acid precipitation procedure (10) using as a substrate 1-*O*-hexadecyl-2-[³H-acetyl]-*sn*-glycero-3-phosphocholine ([³H]-PAF) (10 Ci/nmol; DuPont-New England Nuclear, Boston, MA) at a final concentration of 100 μ M (10). Four μ g of protein from each lipoprotein subfraction or 50 μ l of either plasma diluted 1:50, v/v with HEPES buffer, pH 7.4, or the HDL-containing supernatant after treatment of plasma with magnesium chloride-dextran sulfate (diluted 1:3, v/v with HEPES) were mixed with HEPES in a final volume of 90 μ l and used as the source of the enzyme. The reaction was performed for 10 min at 37°C and PAF-AH activity was expressed as nmol PAF degraded per min per mg of protein or ml of plasma.

Analytical methods

Serum total cholesterol and TG were determined on the Olympus AU560 Clinical Chemistry analyzer (Hamburg, Germany). Serum HDL-cholesterol (HDL-C) levels were measured with the above method in the supernatant, after treatment of serum with dextran sulfate-magnesium chloride for the precipitation of apolipoprotein B (apoB)-containing lipoproteins. Serum LDL-cholesterol (LDL-C) levels were calculated using the Friedewald formula. Serum apoB, apolipoprotein A-I (apoA-I), and apolipoprotein E (apoE) were measured by immunonephelometry with the aid of a Behring Nephelometer BN100 and reagents (antibodies and calibrators) from Behring Diagnostics GmbH (Liederbach, Germany). Serum Lipoprotein [a] (Lp[a]) levels were measured by an enzyme immunoassay method (Macra Lp[a], Terumo Medical Corporation Diagnostic Division, Elkton, MD). The total cholesterol, TG, and phospholipid content in each lipoprotein subfraction were measured by enzymatic methods using the Bio-Merieux kits, whereas free cholesterol was measured by the Boehringer Mannheim kit. The protein content of the lipoprotein subfractions was measured by the bicinchoninic acid (BCA) method (Pierce). The lipoprotein mass of each subfraction was calculated as the sum of the mass of the individual lipid and protein components. Total LDL or HDL mass was calculated as the sum of the mass of corresponding subfractions (10).

TABLE 1. Biochemical and clinical parameters in homozygous and heterozygous FH patients, in polygenic NonFH hypercholesterolemic subjects and in normolipidemic controls

Parameters	Controls	NonFH	HeteroFH	HomoFH	P
Number	33	33	38	5	
Sex (males/females)	14/19	13/20	15/23	1/4	NS
Smokers/non smokers	8/25	10/23	8/30	1/4	NS
Age (years)	40.6 ± 12.9	42.6 ± 5.9	36.5 ± 17.5	23.3 ± 3.1 ^{b,c}	0.01
BMI (kg/m ²)	23.9 ± 1.6	24.8 ± 1.4	24.5 ± 0.8	24.1 ± 1.2	NS
Cholesterol (mg/dl)	199 ± 26	303 ± 49 ^c	340 ± 70 ^{a,c}	448 ± 32 ^{c,f,h}	0.000
TGs (mg/dl)	119 ± 47	141 ± 42	133 ± 41	138 ± 58	NS
HDL-C (mg/dl)	52.2 ± 10.7	50.1 ± 12.7	46.7 ± 12.9 ^{a,d}	36.7 ± 5.7 ^{a,d,g}	0.05
LDL-C (mg/dl)	124 ± 25	222 ± 50 ^c	262 ± 68 ^{c,f}	381 ± 23 ^{c,f,h}	0.000
ApoA-I (mg/dl)	155 ± 21	154 ± 28	143 ± 24 ^{a,d}	101 ± 25 ^{c,f,h}	0.000
ApoB (mg/dl)	136 ± 25	156 ± 29 ^b	182 ± 39 ^{c,f}	229 ± 23 ^{c,f,h}	0.000
ApoE (mg/dl)	33.3 ± 6.9	48.9 ± 8.5 ^c	52.7 ± 13.4 ^c	58.1 ± 23.9 ^c	0.000
Lp[a] (mg/dl)	6.0 (0.8–48.0)	11.5 (0.8–111.0) ^a	11.5 (0.8–42.0) ^a	15.1 (10.7–22.3) ^a	0.006

Values are means ± SD, except for Lp[a] which represent the median (range). NS, not significant.

^{a,b,c} Indicate comparisons with control group, *P* values are <0.05, <0.01, and <0.001, respectively.

^{d,e,f} Indicate comparisons with NonFH group, *P* values are <0.05, <0.01, and <0.001, respectively.

^{g,h} Indicate comparisons with HeteroFH group, *P* values are <0.05, <0.01, and <0.001, respectively.

Statistical analysis

Data were expressed as mean ± SD, except for Lp[a], which was expressed as the median and range. Statistical analyses were performed using ANOVA followed by least significant difference (LSD) test for comparisons between individual groups. Because of the skewed distribution of Lp[a], the nonparametric Mann-Whitney U test and the Kruskal-Wallis test were applied to discriminate for differences of this parameter between groups. Correlations between PAF-AH activity and lipid parameters were estimated using linear regression analysis and Spearman's rank correlation coefficients (for Lp[a]), while Yates's corrected chi-square test was used for differences in proportions. A *P* value of < 0.05 was considered to be significant.

RESULTS

Characteristics of the subjects investigated

Genetic analysis in FH patients participating in the study revealed that all homozygous patients had the exon 12 G(1775)A mutation. Among the HeteroFH patients, 11 had the exon 11 G(1646)A mutation, eight had the exon 12 G(1775)A mutation, seven had the exon 6 C(858)A mutation, six had the exon 9 G(1285)A mutation, five had the exon 2 T(81)G mutation, and one had the exon 4 T(517)C mutation. The characteristics of all hypercholesterolemic patients and normolipidemic volunteers who participated in the study are shown in Table 1. All patient groups exhibited higher levels of serum total cholesterol, LDL-C, apoB, and apoE levels compared with the control subjects. Additionally, all the above parameters were significantly elevated in the HomoFH patients compared with the other patient groups, whereas higher levels were observed in HeteroFH patients, as compared with NonFH subjects. Serum HDL-C and apoA-I levels were significantly lower in the HomoFH patients compared with the other groups. All patient groups displayed higher Lp[a] levels compared to controls, with HomoFH having a significantly higher level than the other two patient groups.

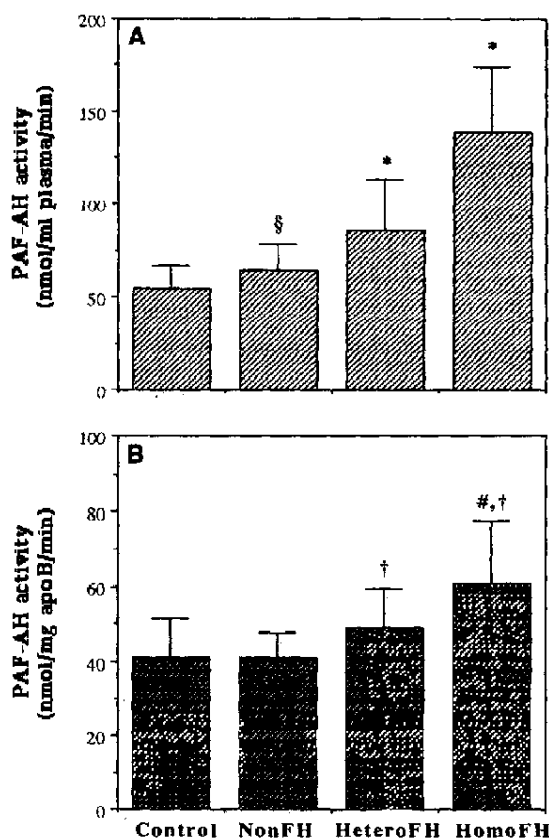


Fig. 1. PAF-AH (PAF-acetylhydrolase) activity in plasma of patients with primary hypercholesterolemia and in normolipidemic controls. A: Expressed per ml of plasma; B: Expressed per mg of apoB. Enzymatic activity was determined by the trichloroacetic acid precipitation procedure using plasma at a final dilution 1:100 (v/v) as the source of the enzyme. Values are the mean ± SD. * *P* < 0.000 compared with all other groups, § *P* < 0.03 compared to controls, † *P* < 0.03 compared to HeteroFH, and ‡ *P* < 0.001 compared with NonFH and controls.

Plasma PAF-AH activity

As shown in Fig. 1A, the total plasma PAF-AH activity (plasma-PAF-AH) in all patient groups was significantly higher than in the control group. Moreover, enzyme activity in HomoFH was significantly elevated compared with either HeteroFH or to NonFH patients. In addition, enzyme activity in HeteroFH was significantly higher than in NonFH patients. When plasma-PAF-AH was expressed per mg of apoB, the same phenomenon was observed except that no difference was observed between NonFH patients and control subjects (Fig. 1B). This finding indicates that the apoB-containing lipoproteins of FH patients are enriched in PAF-AH activity. Plasma-PAF-AH in all study populations was positively correlated with plasma total cholesterol, LDL-C, apoB, and apoE levels, whereas a negative correlation was observed between plasma-PAF-AH and HDL-C, as well as with apoA-I levels (Table 2). When the HeteroFH patients were subgrouped according to the type of mutation, no difference in the enzyme activity expressed either per ml of plasma or per mg of apoB was observed (data not shown).

Unlike the plasma-PAF-AH, the HDL-associated enzyme activity (HDL-PAF-AH), in nmol/ml of plasma/min, was similar among the four groups (3.6 ± 1.1 for HomoFH, 3.9 ± 1.5 for HeteroFH, 3.2 ± 1.5 for NonFH, and 3.4 ± 1.5 for control subjects). As a consequence, an altered distribution of enzyme activity among apoB- and apoA-I-containing lipoproteins is observed in hypercholesterolemic patients, which is characterized by a significant decrease in the ratio of the HDL-PAF-AH to the plasma enzyme activity. This reduction was proportional to the severity of the hypercholesterolemia, being more profound in HomoFH patients (Fig. 2A). A significant decrease in the ratio of the HDL-PAF-AH to the LDL-C levels that was inversely correlated with the severity of hypercholesterolemia was also observed (Fig. 2B). Finally, the same phenomenon was observed in the ratio of the HDL-PAF-AH to the total LDL mass (data not shown).

Chemical composition and mass of individual plasma lipoprotein subfractions

The finding that the LDL of HomoFH and HeteroFH patients was enriched in PAF-AH activity (Fig. 1B) was fur-

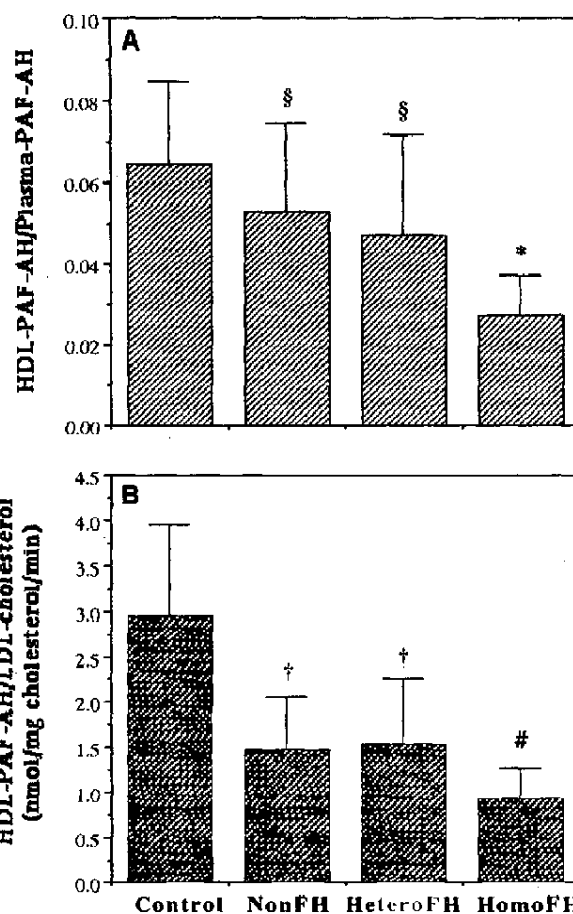


Fig. 2. Bar graph showing the ratio of the HDL-associated PAF-AH activity versus the plasma enzyme activity (A), and versus the plasma LDL-cholesterol (LDL-C) levels (B). Enzymatic activity was determined by the trichloroacetic acid precipitation procedure using either plasma at a final dilution 1–100 (v/v) or the HDL-containing supernatant after treatment of plasma with magnesium chloride-dextran sulfate (at a final dilution 1:6, v/v), as the source of the enzyme. Values represent the mean \pm SD. * $P < 0.01$ compared with all other groups, § $P < 0.05$ compared with controls, † $P < 0.001$ compared with all other groups, and # $P < 0.001$ compared with controls.

TABLE 2. Correlation between plasma PAF-AH activity and lipid parameters

	Plasma PAF-AH Activity			
	Patients		Controls	
	r	P	r	P
Total cholesterol	0.720	0.000	0.535	0.000
HDL-C	-0.205	0.028	-0.274	0.002
LDL-C	0.760	0.000	0.518	0.000
ApoA-I	-0.357	0.000	-0.310	0.001
ApoB	0.760	0.000	0.598	0.000
ApoE	0.340	0.000	0.319	0.000
Lp[a] ^a	0.012	NS	0.03	NS

NS, not significant.

^a By Spearman's rank correlation coefficients.

ther investigated by determination of enzyme activity in individual lipoprotein subfractions. Isopycnic gradient ultracentrifugation was performed in plasma samples containing Lp[a] levels lower than 23 mg/dl (5 HomoFH, 28 HeteroFH, 27 NonFH, and 28 controls) because, as we have previously shown, Lp[a] levels higher than 30 mg/dl significantly affect PAF-AH activity migrated in fractions with density of $d = 1.050$ – 1.100 g/ml, (i.e., between subfractions LDL-5 and HDL-2) (19). No significant differences in the weight percentage chemical composition of corresponding lipoprotein subfractions were observed among the four groups (data not shown). By contrast, total LDL mass (calculated as the sum of the mass of individual LDL subfractions) was significantly higher in all patient groups

TABLE 3. Plasma concentration of lipoprotein subfractions in normolipidemic controls, in polygenic hypercholesterolemic NonFH patients as well as in heterozygous and homozygous FH patients

	Lipoprotein Mass				P
	Controls	NonFH	HeteroFH	HomoFH	
	mg/dl				
VLDL+IDL	44.1 ± 24.1	48.8 ± 7.7	42.3 ± 20.5	39.1 ± 12.3	NS
Total LDL	306.8 ± 107.6	361.6 ± 15.6 ^b	461.5 ± 78.5 ^{c,d}	583.6 ± 64.1 ^{c,h}	0.000
LDL-1	27.6 ± 10.6	34.2 ± 9.1	31.1 ± 13.1	53.2 ± 28.4 ^{a,g}	NS
LDL-2	50.4 ± 26.8	56.3 ± 4.9	72.7 ± 19.2	127.5 ± 44.8 ^{a,i}	0.000
LDL-3	111.9 ± 43.9	131.4 ± 5.9	197.9 ± 26.1 ^{c,f}	244.7 ± 10.3 ^{c,f,h}	0.000
LDL-4	82.5 ± 41.4	103.1 ± 11.6	123.8 ± 27.5 ^b	124.2 ± 8.8 ^b	0.016
LDL-5	34.3 ± 3.6	36.6 ± 15.1	35.7 ± 8.1	33.9 ± 7.9	NS
Total HDL	318.1 ± 74.6	351.1 ± 55.1	234.3 ± 23.9 ^{b,f}	168.5 ± 36.9 ^{a,g}	0.000
HDL-2	113.4 ± 54.2	144.1 ± 40.5	68.1 ± 11.6 ^{b,f}	53.6 ± 27.2 ^{b,f}	0.000
HDL-3	165.1 ± 20.6	172.6 ± 27.7	138.7 ± 12.4 ^{b,e}	91.5 ± 13.3 ^{c,i}	0.000
VHDL	40.8 ± 7.1	34.3 ± 10.9	27.2 ± 5.4 ^c	23.2 ± 6.7 ^{c,d}	0.001

Values are the mean ± SD. NS, not significant.

^{a,b,c} Represent comparisons with control group, P values are <0.05, <0.01, and <0.001, respectively.

^{d,e,f} Represent comparisons with NonFH group, P values are <0.05, <0.01, and <0.001, respectively.

^{g,h,i} Represent comparisons with HeteroFH group, P values are <0.05, <0.01, and <0.001, respectively.

compared with control subjects, with HomoFH having the highest value and the NonFH patients having the lowest one (Table 3). These differences were mainly due to increases in the mass of the LDL-2 and LDL-3 subfractions in each patient group (Table 3). When the mass of each individual LDL subfraction was expressed as percentage of total LDL mass, then LDL-3 was the predominant subfraction in all groups (Table 4). Furthermore, the percentage contribution of this subfraction to the total LDL fraction was significantly higher in the HomoFH and HeteroFH patients. In addition, it was associated with a significant decrease in the proportion of the dense LDL-5 subfraction in these two groups (Table 4).

Total HDL mass in HomoFH and in HeteroFH patients (Table 3) was lower compared with the other groups, with HomoFH HDL mass being significantly lower than that in the HeteroFH patients. This observation is consistent with the lower HDL-C and apoA-I plasma levels in these patient groups (Table 1); such differences arose from the lower HDL mass present in all HDL subfractions (Table 3). Finally, no significant differences were detected in either to-

tal HDL mass or in the mass of individual HDL subfractions between NonFH patients and control subjects. When the mass of each individual HDL subfraction was expressed as a percentage of total HDL mass, HDL-3 was the predominant subfraction; no differences were observed in the percentage HDL subfraction distribution among all studied groups (Table 4).

PAF-AH activity in plasma lipoprotein subfractions

Among the apoB-containing lipoproteins, PAF-AH activity (expressed either per mg of protein or per ml of plasma) was preferentially associated with the dense LDL-5 subfraction in both patient and control groups. As shown in Fig. 3, PAF-AH activity (expressed per mg of protein) in each subfraction was significantly higher in the HomoFH as well as in the HeteroFH patients as compared with that in the corresponding subfractions of the NonFH and control groups. Furthermore, HomoFH patients exhibited significantly higher activity in all subfractions as compared with HeteroFH patients. In NonFH patients, enzyme activity was significantly higher in the LDL-5 and LDL-1, as well

TABLE 4. Mass distribution of individual lipoprotein subfractions in normolipidemic controls, in polygenic hypercholesterolemic NonFH patients as well as in heterozygous and homozygous FH patients

	% of Total Lipoprotein Mass				P
	Controls	NonFH	HeteroFH	HomoFH	
LDL-1	8.5 ± 4.6	9.5 ± 2.8	6.4 ± 1.9	8.8 ± 4.3	NS
LDL-2	17.1 ± 8.9	15.6 ± 1.6	15.5 ± 2.2	21.3 ± 5.4 ^c	NS
LDL-3	36.3 ± 7.6	36.3 ± 1.6	43.3 ± 4.2 ^{a,c}	42.2 ± 3.6 ^{a,c}	0.012
LDL-4	26.1 ± 9.8	28.4 ± 2.2	26.8 ± 3.4	21.6 ± 3.8	NS
LDL-5	12.1 ± 2.9	10.1 ± 3.7	7.8 ± 1.3 ^b	5.9 ± 2.1 ^{b,d}	0.000
HDL-2	33.7 ± 9.6	40.6 ± 7.9	29.1 ± 3.55 ^d	30.8 ± 10.1 ^c	NS
HDL-3	53.3 ± 7.6	49.2 ± 3.9	59.3 ± 2.2 ^{a,d}	55.4 ± 8.5	0.018
VHDL	12.9 ± 3.1	10.1 ± 4.2	11.6 ± 1.8	13.7 ± 2.5	NS

Values are the mean ± SD. NS, not significant.

^{a,b} Represent comparisons with control group, P values are <0.05 and <0.001, respectively.

^{c,d} Represent comparisons with NonFH group, P values are <0.05 and <0.01, respectively.

^e Represent comparisons with HeteroFH group, P value is <0.05.

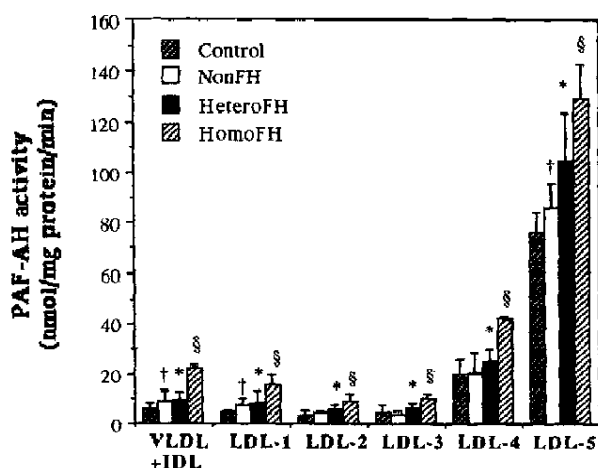


Fig. 3. PAF-AH activity in all apoB-containing lipoprotein subfractions of patients with primary hypercholesterolemia and normolipidemic controls. ApoB-containing lipoproteins were subfractionated by isopycnic gradient ultracentrifugation and enzymatic activity was determined by the trichloroacetic acid precipitation procedure using four μ g of protein from each lipoprotein subfraction. Values represent the mean \pm SD. § $P < 0.001$ compared with values of the corresponding subfraction of all other groups, * $P < 0.01$ compared with values of the corresponding subfraction of NonFH and controls, and † $P < 0.03$ compared with values of the corresponding subfraction of controls.

as in the VLDL + IDL subfraction as compared with their counterparts in the control group. Finally, total plasma enzyme activity in all patient groups and in normolipidemic controls was positively correlated only with the PAF-AH activity associated with both the LDL-4 and LDL-5 subfractions ($r = 0.56$, $P = 0.013$).

In contrast to apoB-containing lipoproteins, no differences were observed in PAF-AH activity (expressed per ml of plasma) associated with each HDL subfraction among all studied groups (data not shown). This finding is in agreement with the observation that all studied groups had similar levels of total HDL-associated PAF-AH activity.

DISCUSSION

Our present study reveals for the first time that PAF-AH activity in plasma, and that specifically associated with LDL particles, preferentially increases, relatively to enzyme activity associated with HDL, in parallel to the increase in the severity of hypercholesterolemia. This relationship was reflected by a progressive decrease in the ratio of HDL-associated-LDL-associated enzyme activity from normolipidemic subjects to NonFH patients, to HeteroFH patients, and to HomoFH hypercholesterolemic patients.

In accordance with previously published results by our group (11) and others (20), the present study suggests that one of the important factors that determines plasma levels of PAF-AH is the rate of removal of LDL from the circulation. Indeed, our results show that the higher

plasma PAF-AH activity in patients with primary hypercholesterolemia is due i) to the elevated number of LDL particles in plasma of these patients, and ii) to the preferential enrichment of dense LDL subfractions with PAF-AH activity. Both observations could be attributed to the lower rate of LDL clearance, which is more profound in the HomoFH and less profound in NonFH patients (21). Consistent with this notion, and in accordance with previously published results (10, 11, 22), is the observation that among the LDL subspecies, PAF-AH is preferentially associated with small-dense LDL. These particles are more slowly removed from the circulation as compared with the larger, lighter LDL particles because of their reduced binding to cellular LDL receptors (23). The preferential association of PAF-AH with small-dense LDL particles is further supported by the present data that reveal that among all LDL subfractions, plasma PAF-AH activity is positively correlated only with that associated with dense LDL-4 and LDL-5 subfractions.

Although the slow rate of LDL clearance could explain both the higher plasma and LDL-associated PAF-AH activity observed in our patients, we cannot exclude the possibility that elevated LDL particle numbers in the circulation of hypercholesterolemic patients could enhance PAF-AH secretion from its cellular sources. Indeed, it is established that PAF-AH secretion from its main cellular sources (macrophages, liver cells) occurs continually, without the need for stimuli (24, 25). Additionally, it has been suggested that the cellular secretion of PAF-AH occurs independently of the secretion of LDL or other lipoprotein particles, and that the enzyme associates with lipoproteins in plasma (26). Consistent with this proposal are previous reports showing that plasma PAF-AH activity is normal in patients with abetalipoproteinemia and is associated with HDL, an indication that LDL is not essential for the existence of normal PAF-AH activity in plasma (27, 28). PAF-AH secretion from macrophages is, however, enhanced when plasma is present (24), and LDL may represent one of the plasma constituents responsible for this effect (Tselepis, unpublished observations). Consequently, the elevated LDL levels typical of hypercholesterolemic patients may influence the secretion of PAF-AH from macrophages. In addition, oxidatively modified LDL contains pro-inflammatory oxidized phospholipids and PAF (5, 29). As reported earlier, the secretion of PAF-AH by liver cells is enhanced in response to stimulation with PAF (30). Consequently, in addition to the macrophage enzyme, the high LDL levels characteristic of hypercholesterolemia may also induce the secretion of PAF-AH from the liver. Based on the above observations, we suggest that the higher enzyme activity per LDL particle found in hypercholesterolemic patients could be due not only to the lower rate of LDL clearance, but also to enhanced secretion of PAF-AH from its cellular sources.

It is well documented that patients with primary hypercholesterolemia exhibit premature atherosclerosis and cardiovascular disease, mainly as a result of high plasma LDL levels (31). Our study shows that the increased plasma-PAF-AH in these patients constitutes a marker of the high plasma LDL levels and is related to the severity of hyper-

cholesterolemia. Thus, plasma-PAF-AH may be considered as a marker of atherogenesis and cardiovascular risk. However, it is not known yet whether the increased PAF-AH activity promotes atherogenesis or whether it represents a defensive mechanism against enhanced plaque formation occurring in these patients. Comparison of data in clinical studies has revealed inconsistent findings concerning the plasma levels of PAF-AH activity in atherosclerotic disease. Thus, some studies have shown an elevation (11, 32), and others have reported lower enzyme activity as compared with controls (33, 34), whereas some studies have failed to detect any difference between patients with atherosclerotic disease and controls (12, 35, 36). Recent results from the West of Scotland Primary Prevention (WOSCOP) study suggest that the plasma levels of PAF-AH mass, which is proportional to its activity and mainly reflects the LDL-associated enzyme, represent an independent risk factor for coronary artery disease (37).

An important observation of our study is that increases in total plasma- and LDL-associated enzyme activity in all patient groups are not associated with any significant change in HDL-PAF-AH. Consequently, a significant alteration in the distribution of PAF-AH between LDL and HDL was observed, resulting in a decrease in the ratio of HDL-PAF-AH to plasma-PAF-AH, which was proportional to the increase in LDL-C levels and consequently to the severity of hypercholesterolemia. A similar phenomenon was observed for the ratio of the HDL-PAF-AH to plasma LDL-C levels or LDL mass, an observation consistent with our previously published data on HeteroFH patients (11). Several lines of evidence suggest that HDL protects LDL from oxidation, and inhibits the biological activity of oxidized LDL. These effects are at least partially mediated by HDL-associated PAF-AH activity (38), which may represent a pool of similar catalytic activities expressed by three different HDL-associated enzymes, PAF-AH, LCAT (39), and paraoxonase (40). The important anti-atherogenic role of the HDL-PAF-AH was further addressed in a recent study in apoE^{-/-} mice, in vivo (41). This approach distinguishes PAF-AH activity associated with atherogenic LDL from that associated with anti-atherogenic HDL. It could also explain the discrepancy between the results of the WOSCOP study showing that the plasma levels of PAF-AH mass is an independent predictor for coronary artery disease (37), and those of other clinical studies, according to which the G⁹⁴→T mutation in the PAF-AH gene that results in a loss of the enzyme activity, could be one of the genetic determinants for atherosclerotic disease in the Japanese population (42, 43). In these studies, the loss in enzyme activity probably reflects not only the LDL-associated enzyme but also HDL-associated PAF-AH activity, the lack of which could possibly be the major determinant for atherogenesis in this population.

In conclusion, our results show that in patients with primary hypercholesterolemia without clinical evidence for coronary artery disease, the plasma-PAF-AH, which mainly reflects the LDL-associated enzyme, is increased without any significant change in the HDL-PAF-AH. This alteration in the relative distribution of PAF-AH among LDL

and HDL results in a decrease in the ratio of HDL-PAF-AH to plasma-PAF-AH, which is proportional to the increase of the plasma LDL-C levels, and consequently to the severity of the hypercholesterolemia. This ratio may be useful as a potential marker of atherogenicity in subjects with primary hypercholesterolemia. ■

This work was supported by research grants from the Greek Ministry of Research and Technology (PENED program 95 ED 656) and the European Community (BIOMED BMH4-CT98-3191 program). We gratefully acknowledge the technical assistance of Dr. M. Vini, Mr. C. Tzallas, and Mrs. E. Lourida.

Manuscript received 17 July 2001 and in revised form 2 November 2001.

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